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Behavioral and Electrocortical Activity in Rats after Neonatal Intraventricular 6-Hydroxydopamine Administration

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NYAKAS, C. AND A. M. L. VAN DELFT. *Behavioral and electrocortical activity in rats after neonatal intraventricular 6-hydroxydopamine administration.* PHARMAC. BIOCHEM. BEHAV. 3(2) 271–277, 1975. – Intraventricular injections of 6-hydroxydopamine (6-OHDA) to newborn rats resulted in a nearly complete disappearance of catecholamines in brain regions containing nerve terminals. In the hypothalamus, however, dopamine was only decreased to 60% of control levels. The exploratory (rearing and crossing) and some of the automatic type behavioral activities (grooming, chewing and gnawing), the duration of neocortical synchronization and hippocampal theta frequency were measured during light and dark periods in adulthood. Decreased exploratory and increased automatic behavioral activities were found in both light and dark periods and a high incidence of neocortical synchronization and a lowered hippocampal theta frequency in the light phase when the animals were repeatedly subjected to a strange environment. The light–dark rhythmicity of exploratory and hippocampal activity remained intact in the 6-OHDA treated rats. Furthermore, the diurnal periodicity of general motor activity was also normal. It was concluded that contrary to the active involvement of prepontine catecholamine structures in the behavioral and electrocortical responses in a novel situation, their role in controlling the light–dark rhythmical processes may not be essential.

Exploratory activity Automatic type behavior Hippocampal theta frequency Neocortical synchronization
Light–dark rhythms Catecholamines 6-Hydroxydopamine Neonatal treatment

THE role of catecholamines in central neuronal circuits and of dopamine in nigro-striatal pathways in particular have been studied extensively with respect to the regulation of motor responses or fixed motor patterns [2,30]. Detailed experimental analysis has been performed on involvement of catecholamines in behavioral arousal [2,15] and electrocortical and sleep mechanisms [12, 15, 22]. A longlasting and specific destruction of catecholaminergic nerve endings in the mammalian brain can be achieved by central administration of 6-hydroxydopamine (6-OHDA) to both adult [3,31] and newborn rats [4, 6, 16, 20, 22, 27]. Following neonatal 6-OHDA administration a marked deficit has been found in body growth and consummatory behavior [4, 6, 22, 27], furthermore evidence for a blockade of shuttlebox avoidance acquisition [22,27], and a dose dependent decrease in exploratory activity [27] has been reported.

Exploratory activity in rats is a characteristic behavioral response to a change in the surrounding environment. It is known that during exploratory behavioral movements (voluntary movements) such as rearing, walking around and sniffing, the hippocampus shows a rhythmical slow electrical activity (theta rhythm), while during another more automatic group of behavioral movements like face-washing, scratching, licking, chewing, gnawing, the hippocampal electrical activity is irregular and does not show the characteristics of the theta rhythm [1, 24, 32, 33].

In the present paper we attempted to further investigate rats largely depleted of their central catecholamines by neonatal 6-OHDA administration. The responsiveness of the animals to a novel environment was studied in respect to exploratory and automatic type behavioral movements classified on the basis of the appearance or absence of hippocampal theta rhythm, and to neocortical and hippocampal electrical activity in general. Because of the important circadian variations in the various parameters, studies were done both during the light and dark cycle.

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METHOD

Animals, Drug Dosage and Surgery

Newborn inbred Wistar male rats were randomly distributed over mothers and each litter, containing 7–9 pups was injected intraventricularly with 6-hydroxydopamine hydrochloride (6-OHDA, Labkemi AB, Göteborg) or vehicle. The schedule of injection of 200 μg of 6-OHDA was the following: increasing doses of 40, 60, and 100 μg were injected into the lateral ventricles slowly throughout a 5 min period on Days 2, 4 and 6 after birth, respectively, in a volume of 5 μl of 0.1% ascorbic acid solution with a technique described earlier [22]. Dry food and water were given ad lib and during weaning at the age of 25 days, a wet mash of food pellets was available additionally in order to maintain continuous body weight gain in the treated rats. The animals were weighed 3–4 times per week and this procedure served as habituation of animals to handling and to the presence of the experimenter. The animals were kept under a lighting schedule of 14 hr light (from 7 a.m. till 9 p.m.) and 10 hr dark throughout their life.

Surgical procedures were carried out at 10 weeks of age under sodium-hexobarbital (100 mg/kg) anaesthesia. Teflon insulated stainless steel electrodes were implanted into the dorsal hippocampus and the surface of the contralateral frontal cortex. Bipolar hippocampal electrodes were inserted stereotaxically in antero-posterior direction 1 mm apart from each other into the region of the pyramidal layer in the CA_1 area with the coordinates of 1.8 mm lateral, 3.2 and 4.2 mm anterior and 3.0 mm deep from the surface of the parietal bone according to the atlas of König and Klippel [18]. From the electrodes only the cut off surfaces of their tips remained uninsulated; they were fixed to the bone by means of 2 stainless steel screws and acrylic cement (Simplex, Dental Fillings Ltd., London).

Behavioral and Electrophysiological Procedures

The effect of 6-OHDA administration on exploratory activity was tested in a 12 compartment exploratory maze [8] both before and after onset of puberty during 3 daily sessions of 5 min each. The number of crossings through the gates between the compartments and the number of rearings were recorded.

At 6–7 days after the electrode implantation the rats were tested in a simple Plexiglas box measuring 30 × 38 × 30 cm as a novel environment. The floor of the box was covered like the home cage with wooden shavings and several food pellets. The behavioral and electrocortical recordings, taken between 1 and 3 hr after the start of a light or dark period, were performed throughout 5 consecutive light and dark sessions. A red lamp served for illumination during observation in the dark period.

Of the different elements of exploratory activity which are accompanied by rhythmical theta activity [24] only the number of rearings was counted. In order to measure the automatic type behavioral activities, not associated with the appearance of hippocampal theta rhythm, but with large amplitude irregular activity [1,33], the total duration of grooming (washing the face, scratching, licking different parts of the body), chewing (food or pieces of wood) and gnawing was measured. Each rat was placed individually into the box for 20 min. During the first 10 min the behavioral parameters were observed and during the second 10 min the electrocortical activities were recorded on a 4 channel Hellige polygraph (Programm 19) by means of a noiseless cable connected through Amphenol plugs with the chronically implanted electrodes of the freely moving animals. The continuous exploratory movements (rearing and walking around with sniffing) were marked manually on the EEG recordings. The duration of neocortical synchronization recorded from the frontal cortex and the hippocampal theta frequency were evaluated visually. The criteria of the calculation of hippocampal theta frequency were developed in order to get a single parameter of hippocampal EEG activity which is characteristic for the intensity of the exploratory activity in a novel environment. Considering that any arrest or pause in the continuity of the gross exploratory movements mentioned above blocks the theta train, only hippocampal theta fragments of 1 sec duration were selected from the very beginning of theta recordings marked under continuous exploratory movements. Thus the individual values of hippocampal theta frequency were calculated following each session as the mean of 10 samples of theta trains of 1 sec duration obtained during the first 10 consecutive exploratory movements.

At the end of the experiments the circadian rhythm of general motor activity was measured by an Animex activity meter in both 6-OHDA treated and vehicle injected paired rats. This was done after 12 hr habituation to the experimental situation. Evaluation of a 24 hr period was on the basis of counts printed every hour.

Biochemical Procedures

Five to seven days after the last behavioral session the animals were killed by decapitation, the brains were rapidly removed and dissected in a cold room into the following parts: (1) lower brain stem (pons and medulla), (2) mesencephalon, (3) hypothalamus (preoptic area included), (4) cerebellum, and (5) rest of brain (telencephalon and thalamus) as illustrated in Fig. 1.

Norepinephrine (NE) and dopamine (DA) content were assayed by the method of Shellenberger and Gordon [26] in pooled sections from 2 or 3 rats.

RESULTS

The content of NE and DA in different parts of the 12 weeks old rat brain following neonatal 6-OHDA treatment is shown in Fig. 1. The destruction of catecholaminergic structures in the most frontal part of the brain was almost complete. NE depletion in the mesencephalon and hypothalamus was also quite prominent. No change was obtained in NE content of the lower brain stem. A statistically significant change was observed in the hypothalamic DA content, but the depletion was about 40 per cent.

The number of crossings and rearings in a 12-compartment exploratory maze which were measured before and after puberty are shown in Fig. 2. 6-OHDA treated rats proved to be inferior in both kinds of exploratory activity in both ages (Student's t test $p<0.01$ for crossings during Days 31–33, and $p<0.001$ for all other comparisons). No tendency for habituation could be observed in the course of the 3 daily 5 min sessions. The reduction in crossing activity appeared to be considerably less pronounced than that in rearing activity, especially at an earlier age (Days 31–33).

In the next experimental series the rats with chronically implanted electrodes into the dorsal hippocampus and

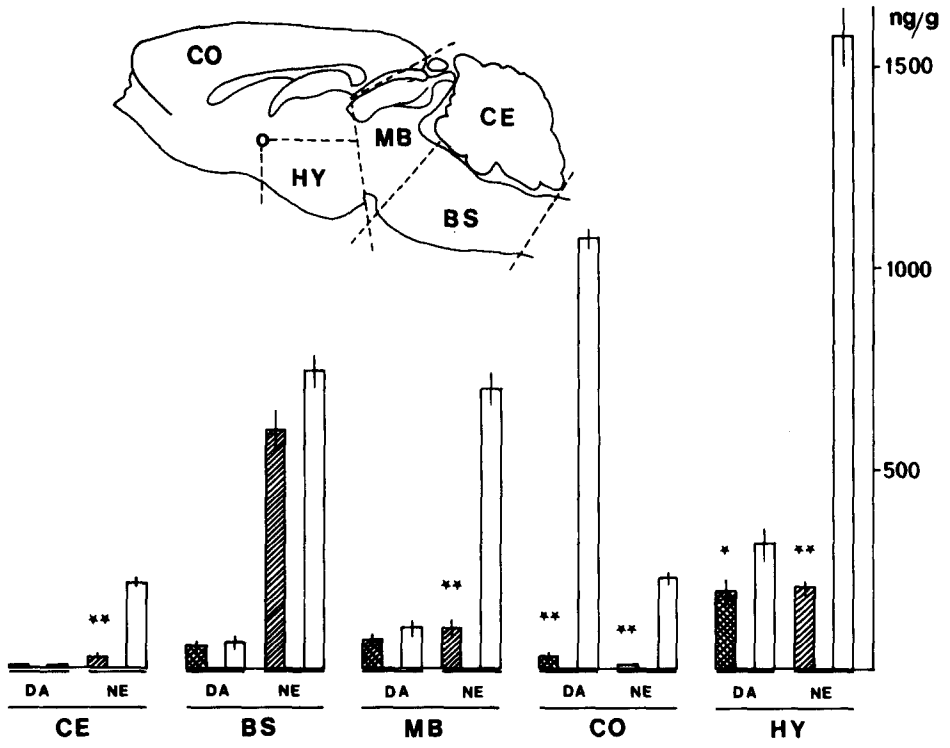

FIG. 1. Effect of 200 μg 6-OHDA administration in the first week of life on norepinephrine (NE) and dopamine (DA) content of different parts of the 13 weeks old brain. Mean ± S.E.M. of 6–10 samples are presented. BS = brain stem (including pons and rostral medulla); CE = cerebellum; MB = mesencephalon; HY = hypothalamus (including preoptic area); CO = rest of the brain. Open bars controls, hatched and crossed bars 6-OHDA treatment. Significant differences were calculated by Student's t test (*$p<0.05$; **$p<0.01$).

frontal cortex were tested for exploratory activity in a one compartment exploratory box, and only the rearings were counted during 10 min sessions in the light or dark periods. Together with the counting of rearing activity the total duration of grooming, chewing and gnawing as manifestations of automatic type behavioral activities were measured. The results are summarized in Fig. 3. As in the preliminary test in the exploratory maze, the number of rearings in 6-OHDA treated rats, again was markedly reduced during the light session (Student's t test $p<0.001$) and also during the dark sessions ($p<0.001$). Automatic behavioral functions showed a marked increase in the 6-OHDA treated rats in light and dark sessions ($p<0.001$ in light and dark sessions). Both the treated and vehicle injected groups showed a light–dark rhythm in rearing activity (controls and 6-OHDA treated rats: $p<0.001$, t test for related samples), which could not be observed for the automatic type behavioral activity. In fact, a shift from the exploratory activity towards the automatic types of behavior can be postulated as a result of treatment during exposure to a strange environment. It may be mentioned that when grooming and eating times of 10 sessions were calculated separately both kinds of automatic behavioral activities were elevated in treated versus control rats (grooming: $p<0.001$; chewing and gnawing: $p<0.02$).

Immediately after the observation period of 10 min for assessing the intensity of exploratory versus automatic type behavioral activities, the electrocortical, i.e. hippocampal and neocortical activities, were monitored for another 10 min period. Data are shown on the right side of Fig. 3. In sharp contrast to control rats 6-OHDA treated rats frequently showed drowsiness and occasionally sleeping-like behavior accompanied by neocortical synchronization during the 10 min testing period in light and dark. Statistical evaluation of the duration of neocortical synchronization in 6-OHDA treated animals showed that despite this abnormal behavior, light–dark differences still persist ($p<0.01$, t test for related samples). The theta frequency during continuous gross exploratory movements (rearing, walking around and sniffing) was lowered during the light sessions ($p<0.02$) but not in the dark ($p<0.05$). Both groups of animals showed a clear light–dark rhythm in the hippocampal theta frequency (6-OHDA and controls: $p<0.001$). In both groups of animals a higher hippocampal theta frequency appeared during exploration at night. Fig. 4 shows examples of neocortical synchronization found in 6-OHDA treated rats. Quite frequently the 6-OHDA treated rats showed synchronized EEG trains between small exploratory movements like head turning and sniffing (see Fig. 4, lower recording), but during the neocortical synchronization the rats were motionless. In several cases during neocortical synchronization of short duration the eyes remained open, but also drowsiness or sleeping-like activity could be observed in treated rats.

General motor activity (both exploratory and automatic) which was measured throughout 24 hr by an

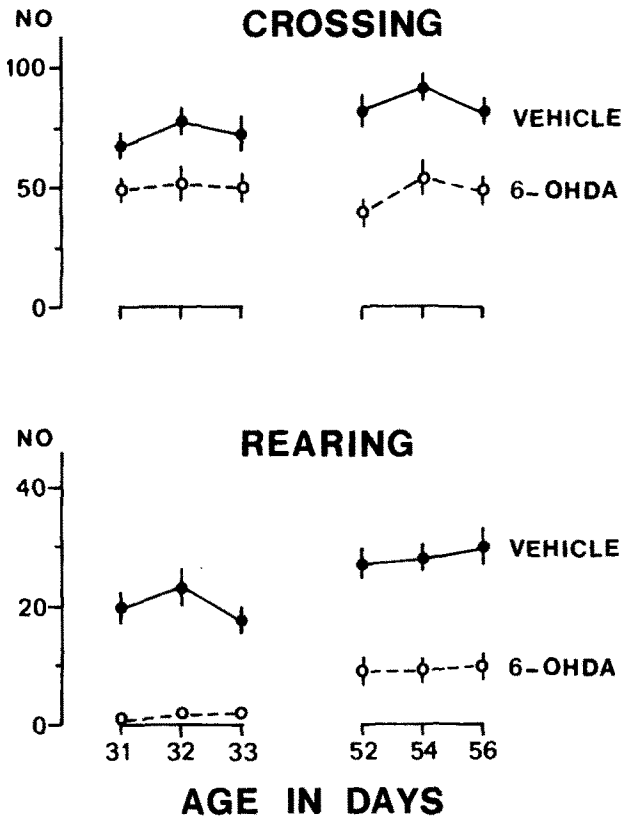

FIG. 2. Number of crossings and rearings in a 12 cell exploratory maze as estimated before and after puberty following central injection of 200 μg 6-OHDA in the first week of life. Mean ± S. E. M. of 7 control and 9 6-OHDA treated rats are shown. Each day significant ($p<0.01$) differences between control and 6-OHDA were found.

Animex recorder, shows the same circadian pattern in treated as in control rats (Fig. 5). The only exception was at 20 hr and 21 hr p.m. where the 6-OHDA treated rats showed significantly less activity (20 hr: $p<0.05$; 21 hr: $p<0.02$). This moderate difference may be considered as a delay in the initiation of increased motor activity induced by darkness.

A body weight retardation of 35–36 percent was found in the treated rats after the weaning period which did not change further in adulthood (± 2 per cent). This prominent retardation of the body weight may be explained by a decreased food intake occurring predominantly during the weaning period. It may be noted here that in an earlier study we had been unable to find any change in behavior as a consequence of the decreased body weight [22]. A hypersensitivity to handling (biting, vocalization) of the treated rats was present even after the regular habituation procedure.

DISCUSSION

After intraventricular injections of 200 μg 6-OHDA to newborn rats a nearly complete and irreversible loss of NE was found in the prepontine brain regions and of DA in the forebrain. However, the NE content of the lower brain stem remained unchanged, and the DA content in the brain stem and hypothalamus showed only moderate changes. Histochemical fluorescence studies in our laboratory (Tilders, unpublished data) however, revealed marked regional differences: for example, catecholamine fluorescence in the region of the median eminence remained intact in these rats contrary to other parts of the brain like in the lateral hypothalamic region and cortical areas where the disappearance of fluorescent material was almost complete.

The marked decrease found in exploratory activity, and especially in rearing, confirmed our earlier results with 6-OHDA [22]. Among the different forms of exploratory activity, rearing has been claimed to be highly correlated with the nonspecific excitability level in the brain [19]. Histofluorescence studies [7,9] demonstrated that the dorsal and ventral noradrenergic pathways might have some anatomical relation with the neocortical and limbic arousal systems [25]. Therefore, these considerations would support the idea that NE may play a role in the mediation of arousal, learning and memory function [17]. However, DA has been implicated by others, as the neurotransmitter involved in behavioral arousal and avoidance behavior [2, 15, 27]. It should be emphasized here that in the discussion on the involvement of NE and/or DA it is necessary to differentiate between different varieties of behavioral arousal (awakeness, consciousness, exploration, conditioned behavior). Specific involvement of NE or DA in these behavioral varieties is the subject of current research [15, 27, 29]. Smith *et al.* [27] reported that preferentially depleted DA by 90 per cent in newborn rats could mimic the effects of both DA and NE depletion in body growth, consummatory behavior, locomotor activity and shuttle box avoidance while preferential depletion of NE by 45 per cent showed controversial effects on these behavioral variables.

In the present study an increase in automatic type behavioral activities was observed suggesting effects of 6-OHDA on the appearance of these types of behavior opposite to that seen in exploratory behavior. This may be of interest because data from hippocampal research [1, 24, 32, 33] indicate that electrocortical activities occurring during exploratory versus more automatic movements are derived from separate neuronal circuits. It has been assumed that automatic behavioral activities are related to nonspecific activation [13] as found for example after stimulation of the posterior or lateral hypothalamus. This stimulation evokes organized motor patterns such as eating [14], gnawing [23] and copulation [5] which have reinforcing properties [10]. Involvement of DA through the nigrostriatal pathways has been claimed recently in these behavioral activities [2,30]. It can be assumed that following neonatal 6-OHDA treatment, the striatal dopaminergic receptors remain intact [6]. Involvement of these possibly supersensitive dopamine receptors in the increase of stereotype like automatic motor patterns might therefore be accepted as one of the factors responsible for the augmentation of automatic behavioral activities. As a working hypothesis, it may be assumed that the exploratory activity and automatic activities are inversely related to each other, possibly through reciprocally connected neuronal inhibitory processes.

The physiological meaning of hippocampal theta activity is not clear, despite extensive studies made especially under conditioned behavioral circumstances. It was mentioned that exploratory activity but not automatic type behavioral movement is accompanied by regular and frequent theta activity. From our data we may claim that an extensive damage of the catecholaminergic terminals, especially in the

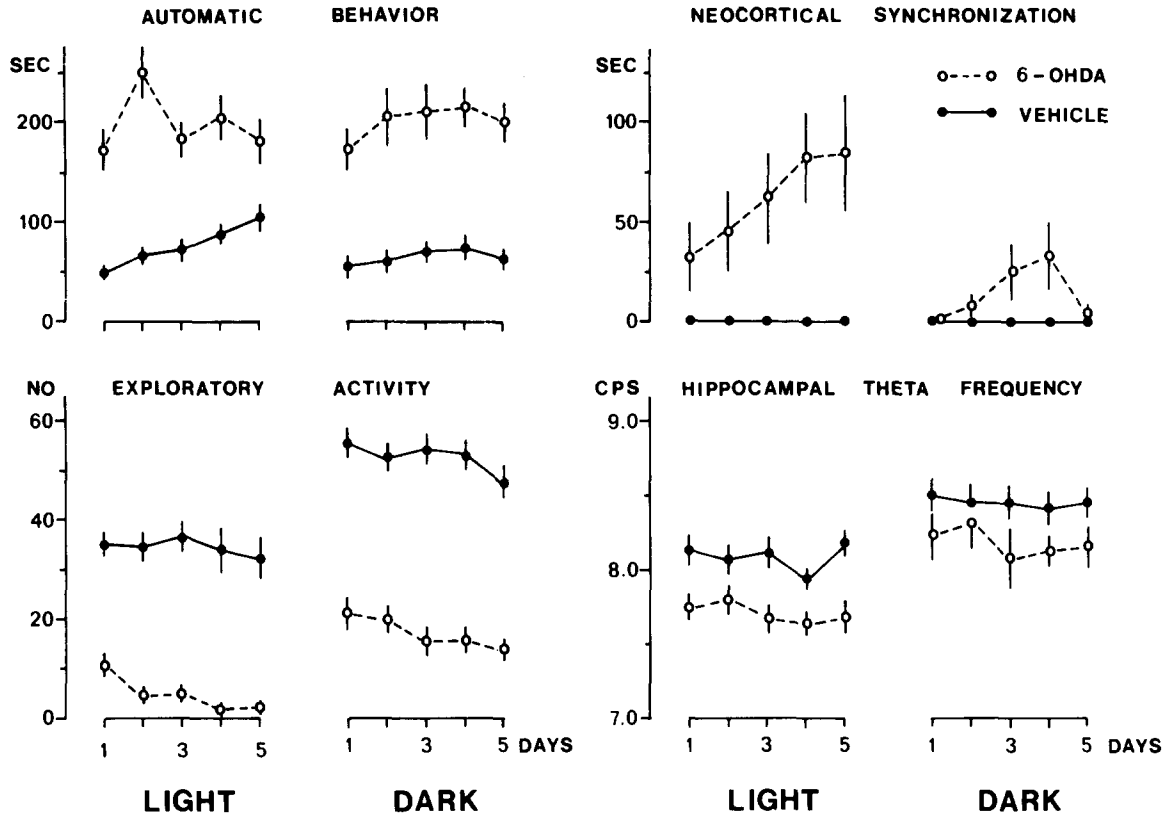

FIG. 3. Behavioral and electrocortical correlates of neonatal 6-OHDA treated rats placed in a novel environment during 5 consecutive sessions in light and dark periods. Duration in seconds of automatic behavior (grooming, chewing, and gnawing) and the number of rearings for exploratory behavior in the course of the first 10 min observation period are presented on the left. During the next 10 min the duration of neocortical synchronization and hippocampal theta frequency during exploratory movements were measured. These data are shown on the right side of this figure. Means ± S.E.M. of 13 control and 11 6-OHDA treated rats (9 for EEG data) are presented. See text for significant differences.

basal forebrain and the cortical areas does not grossly interfere with the appearance of normal theta rhythm, i.e. it does not influence those neuronal mechanisms which are responsible for the generation of theta activity through the medial septal area [28]. This result is in agreement with those found after 6-OHDA treatment in adult rats [11]. Evaluation of theta frequency measured during continuous exploratory activity showed that 6-OHDA and vehicle injected groups increased theta frequency during the dark period, as compared to the light period. This increase of hippocampal theta frequency in darkness may be due to the increase of the intensity of exploration activity. On an absolute basis, however, theta frequency was lower in the 6-OHDA treated rats during exploratory activity in a novel environment (particularly in the light period). A sharp distinction between the exploratory and the stereotyped locomotor activity would be helpful to explain this difference.

The appearance, within a relatively short period, of neocortical synchronization in 6-OHDA treated rats placed in a novel environment was a very characteristic phenomenon in our study. This phenomenon might be correlated with a marked decrease in rearing found in the course of daily light sessions. Neocortical synchronization during the dark sessions was much less pronounced than under light conditions. Recently [11,21] temporary changes were also found in both desynchronized and slow wave sleep following large dose of 6-OHDA injected intracisternally or intraventricularly into adult rats, confirming some of our results.

The light–dark rhythm of exploratory activity and the hippocampal theta frequency appeared to be still present in the 6-OHDA treated rats. No considerable change was found in the circadian rhythm of general motor activity. In our earlier paper [16] it was reported that neonatal 6-OHDA administration did not interfere with the diurnal control of the pituitary-adrenocortical system, which supported the conclusion that brain catecholamines, at least the prepontine noradrenergic and the forebrain dopaminergic structures, are not essential in regulating light–dark rhythms. The present results demonstrate that catecholaminergic nerve endings in the rat brain are of minor importance for the generation of some rhythmic processes but are important for behavioral and electrocortical responses in a novel situation. Moreover, they seem to be essential for the generation of fully organized exploratory activity.

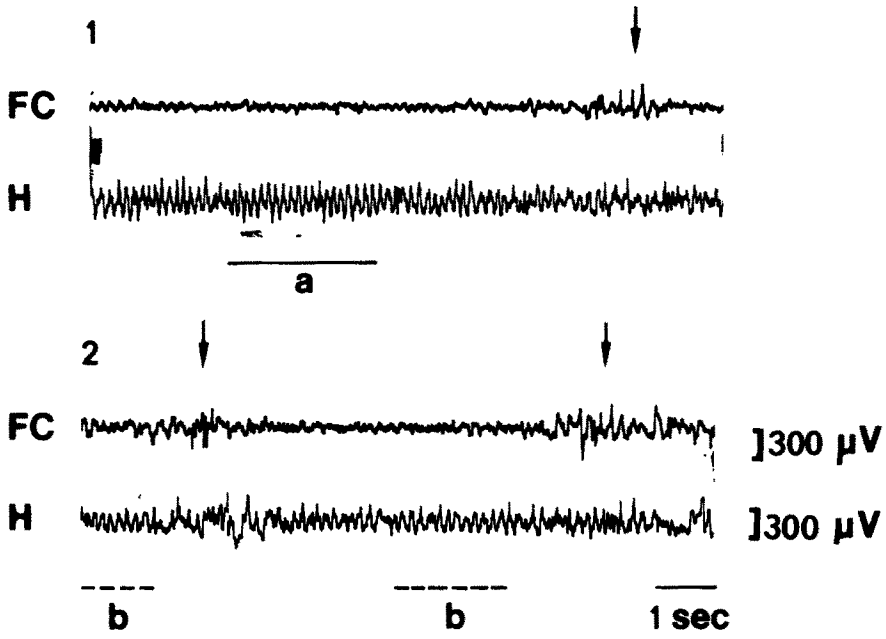

FIG. 4. Electrocortical recordings from the frontal cortex (FC) and dorsal hippocampus (H) in an adult rat injected with 6-OHDA neonatally. The arrows indicate the appearance of neocortical synchronization in the novel environment. Rearing is marked by a continuous line (a), while head turnings are marked by broken lines (b).

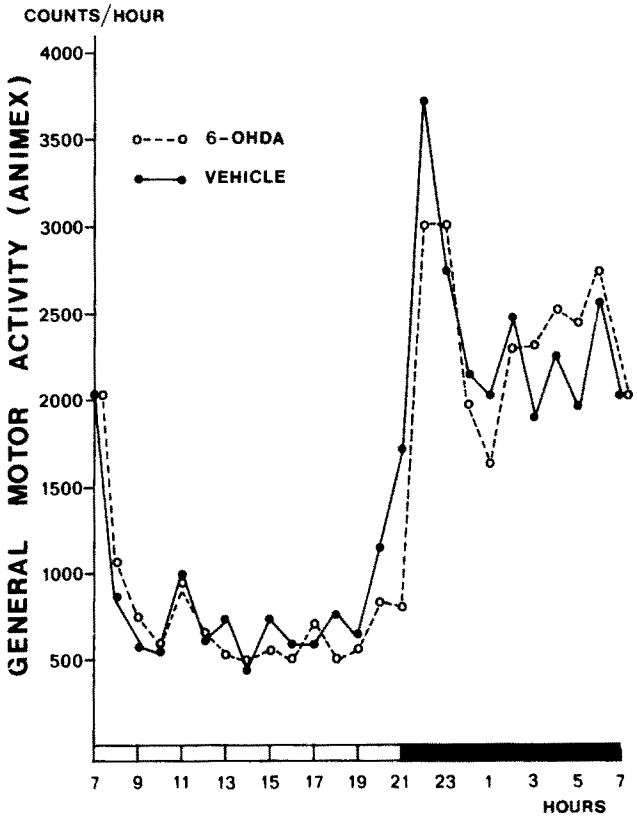

FIG. 5. Circadian periodicity of general motor activity in neonatally 6-OHDA treated and vehicle injected 12 weeks old rats. Recording started 12 hr following the placement of paired subjects into the activity cage (Animex). Mean activities of 9 6-OHDA treated and 7 vehicle injected pairs are shown.

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